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Art. I.—*Notes on the Histology of the Alimentary Canal in some Australian Termites.*

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(With Plates I. and II.)

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Introduction.

Observations on the alimentary canal of Metatermitidae of the genus *Eutermes* show several peculiar features which seem to have been overlooked in the most complete previous account, that of Holmgren (1909). It is the purpose of this paper to recapitulate Holmgren's description, to describe the structures which he did not mention, and to compare them with analogous structures, if present, in termites of other families.

The classification adopted is that of Holmgren.

The order Isoptera is divided into four families:—

Mastotermitidae (type *Mastotermes*), Protermitidae (*Archotermopsis, Calotermes, Porotermes, Stolotermes*). Mesotermitidae (*Coptotermes*), and Metatermitidae (*Eutermes*).

Method.

Longitudinal and transverse serial sections, usually 10μ thick, were cut of alimentary canals dissected out and fixed in Bouin or corrosive acetic. Delafield's haematoxylin and Heidenhain's iron haematoxylin, with or without an eosin counterstain, were the most generally satisfactory stains; occasionally picric acid was used to show chitinous structures. Unstained alimentary canals mounted whole were useful for observing the demarcation of the various regions.

General Features of Alimentary Canal.

The alimentary canal of a termite may be divided externally into the following regions:—

(1) *Fore-gut*, consisting of pharynx, oesophagus, crop and gizzard. Posteriorly the fore-gut may be invaginated into the mid-gut, the invaginated portion and the tube immediately anterior to it being termed by Holmgren the "collum" and by Imms (1919) the "oesophageal valve."

(2) *Mid-gut*, the posterior boundary of which is marked by the opening of the Malpighian tubes.

(3) *Hind-gut*, which can be divided into five parts:—

(*a*) A narrow tube of varying length, extending from the end of the mid-gut to the posterior curvature. This division is particularly long in Metatermitidae, but short in the other three families.

(*b*) The region extending from the posterior curvature to the greatly dilated "large intestine" of Imms. The walls of this part of the gut are very muscular, deeply folded, and bear numerous chitinous spines. The great development of muscular coats suggests that the final subdivision of food occurs here; this region which is called by Imms the "pyloric valve" has been termed in this paper the *posterior gizzard*.

(*c*) The large intestine named by Holmgren the "Hinterdarmblase" is a large thin-walled sac projecting to one side of the intestine.

(*d*) A narrow tube leading from the large intestine to the slightly expanded rectal portion.

(*e*) Rectum.

As types of the family Metatermitidae *Eutermes dixoni,* and *E. exitiosus,* which very closely resembles it, have been used.

These species agree in most particulars with *Eutermes chaquimayensis* as described by Holmgren, but show some interesting and characteristic structures which are not mentioned in his account.

Macroscopic Structure of Genus Eutermes.

In the genus *Eutermes,* the pharynx is short. The oesophagus extends through the thorax to a dilated conical crop, behind which lies the gizzard. This is lined by large crushing plates, clearly visible in alimentary canals which have been dissected out and mounted whole.

The mid-gut is long, tubular, and U-shaped. From it, at the point of entry of the four Malpighian tubes, leads the hind-gut.

The slender anterior part (= section (*a*)) of the hind-gut is elongated in *Eutermes,* and forms a large loop passing behind the posterior end of the crop. The dilated large intestine, which immediately follows the tubular anterior portion, forms one limb of another loop. It bends back on itself, and is continued as the tubular hind part, which becomes expanded at the posterior end to form the rectum, and opens to the exterior by the anus.

Histology of Eutermes dixoni and E. exitiosus.

Fore-Gut.

The *fore-gut* is similar to that of *E. chaquimayensis.*

The *oesophagus* consists of cubical epithelium, beneath which lies an outer circular and an inner longitudinal muscle layer.

The *crop* has the same two layers as the oesophagus, but its lumen is much dilated.

The *gizzard* is characterized by continuous longitudinal ridges used for grinding food. These ridges are heavily chitinized, and are of two types:—

(1) Tall narrow folds, constricted at the base, which appear club-shaped in transverse section.

(2) Lower cushion-like folds over which the chitinous lining is thinner.

Each ridge contains a core of connective tissue covered by columnar epithelium which secretes the chitin. The edges of the tall ridges are finely serrated. There is a well-developed circular layer of striated muscle.

The *œsophageal* valve or collum, which projects from just below the gizzard into the mid-gut, is composed solely of thin columnar cells resting on a basement membrane.

Mid-Gut.

The wall is slightly folded, and is composed of cells arranged in three ways:—

(1) An outer thin epithelium of flattened cells.

(2) Elongated columnar cells, which are broader at their outer edge, and have a striated border, a large nucleus and definite nucleolus. They have the appearance of glandular tissue.

(3) Crypts of cells, lying at right angles to the long axis of the mid-gut. These vary in number and in depth in different specimens. It is probable from their great variability that they are nests of young cells from which the epithelium is regenerated, as suggested by Imms, rather than glandular crypts as assumed by Holmgren. In some sections the crypts are so numerous and deep that the gut wall has a "scalloped" appearance, while in surface view the wall shows a mosaic effect.

The muscle-layers are very poorly developed. The cells seem to be bounded on the outside by a thin membrane only.

Malpighian Tubes, four in number, are lined by a single layer of flattened epithelium, the cells of which frequently contain refractive granules.

Hind-Gut.

(Pl. I., Fig. 1F; Pl. II., Figs. 2A, B, and 3A, B, C, and D.)

Eutermes dixoni and *E. exitiosus* are similar to *E. chaquimayensis* in many ways, but show some conspicuous features not included in Holmgren's description.

Division (*a*) of the hind-gut is very much elongated, and has down one side for half its length a strip of cell continuous with and similar to those forming the mid-gut wall, i.e., apparently glandular cells interspersed with regenerative crypts. This

structure extends from the mid-gut to a point half-way between the opening of the Malpighian tubes and division (*b*)—the posterior gizzard.

Except for this strip the wall of this division of the hind-gut is composed of a thin layer of flattened epithelium.

Running longitudinally on each side of the strip is a fold or valve (Fig. 2A). The tissue composing this forms a nearly complete ring at the level of the Malpighian opening (Fig. 3A and B), and then is continued backwards as two projecting folds (Fig. 3C). Anteriorly the folds project inwards towards the middle line. Further back they meet and fuse (Fig. 3D), thus enclosing a space between them and the strip of glandular tissue. No food has ever been observed in this space, even when the main cavity of the gut is full of food.

Each fold consists of two layers (Fig. 2B) :—

(1) An inner flattened or cubical layer on the side towards the glandular strip.

(2) An outer layer of deep columnar cells with clear protoplasm and distinct nuclei.

The Malpighian tubes open above the origin of the valve, i.e., into the posterior end of the mid-gut.

The region of the hind-gut leading from division (*a*) to the large intestine is very characteristic. It is here termed the *posterior gizzard* (division (*b*)) since in *Eutermes*, as in other genera, the folded muscular walls have a chitinous lining bearing numerous pointed toothlike projections (Fig. 1F). The lumen of this region is narrow and the walls are very muscular. It is probable that it serves to grind any larger particles of food which have been left by the anterior gizzard before they enter the large intestine. Imms working on *Archotermopsis*, names this region the "pyloric valve," and holds that the backwardly-pointing teeth serve to prevent the regurgitation of large particles into the mid-gut. He considers that the fluid contents of the hind-gut can be regurgitated, and are acted upon by the secretion of the mid-gut. Grassi and Sandias (1895) held a similar view, and termed the mid-gut the "chylific ventricle."

Holmgren does not mention the presence in *Eutermes chaquimayensis* of either double valve or mid-gut strip, neither does he record the presence of chitinous teeth in the posterior gizzard region, although he describes "lashes" in the large intestine itself. The writer has seen no constant structures corresponding to his "lashes," but occasionally chitinous threads can be observed stretching from the tip of some of the folds to the food. In other respects the histology of the "large intestine" of *Eutermes dixoni* and *E. exitiosus* is similar to that which Holmgren describes for *E. chaquimayensis*. The more slender part of the gut behind the large intestine and the dilated rectal portion are also similar to those of *E. chaquimayensis*.

Comparison between certain Structures of the Hind-Gut in Eutermes and in Termites of other Families.

1. Mastotermitidae.

Mastotermes darwiniensis (Figs. 1A, 4).—The wall of the first division of the hind-gut is thrown into longitudinal folds made up of columnar epithelium supported on a pad of connective tissue. For a short distance anterior to the gizzard, the wall bears fine spines pointing forwards. Although there is a fair development of muscles here, they are much less marked than in the posterior gizzard itself.

The posterior gizzard bears four deep longitudinal folds, which are supported by well-developed outer circular and inner oblique muscle layers. In some sections the oblique muscles seem so disposed that one fibre goes to each epithelial spine-bearing cell, suggesting independent movement of the individual teeth. The lining epithelium is composed of short columnar cells, bearing large backwardly-directed spines.

From the gizzard to the large intestine there extend four shallower folds, the chitinous lining of which bears spines like those of the gizzard. The actual spine-bearing surface is greater in *Mastotermes* than in any other termite which the author has examined.

2. Protermitidae.

In termites of this group, as well as those of the Mastotermitidae and Mesotermitidae, the first division of the hind-gut is not elongated, i.e., the Malpighian tubes arise comparatively near the large intestine.

Archotermopsis wroughtoni.—The histology of the alimentary canal of this termite was fully described by Imms (1919).

> "The first portion of the hind-gut is 0.9-1 mm. in length. . . . . Near the middle of its course is a group of three inwardly-projecting folds, covered by a chitinous lining, and invested with fine backwardly-directed spines. . . . These folds are well-supplied with muscle-fibres, and serve as a valve. . . . At the junction with the large intestine, four groups of columnar epithelial cells pass back from the valve region, and project for a short distance into the cavity of the large intestine."

Imms states that the folded walls of the gut, both anterior and posterior to the posterior gizzard or "valve" have a chitinous lining, but he does not mention the occurrence of spines in either position.

Calotermes insularis (Fig. 1B).—Posterior to the opening of the Malpighian tubes, the hind-gut wall is thrown into longitudinal folds. Anteriorly these consist of columnar epithelium, but this changes to cubical epithelium, i.e., the folds become lower, towards the posterior gizzard.

The portion of these folds next to this gizzard bears, for approximately one-third of the total length, small chitinous teeth which point in the opposite way to those in the gizzard itself. These latter are developed on continuous, rather shallow longitudinal folds, which lead directly into the large intestine. The muscle-layers are not so easily defined as those of *Mastotermes.*

Stolotermes victoriensis (Fig. 1c).—The hind-gut of *Stolotermes* shows a condition intermediate between that found in *Calotermes,* and that of the more highly-developed *Porotermes.* The walls are thrown into folds which are shallower and more uniform than those of *Porotermes,* and are lined by low columnar epithelium. The posterior gizzard is very muscular, the arrangement being similar to that previously described in *Mastotermes.* The chitinized epithelium of the posterior gizzard has large spines, but there are no spines on the folds anterior to it as were described in *Calotermes,* and no toothless folds posterior to it.

Porotermes grandis (Figs. 1d, 5).—Immediately posterior to the entry of the Malpighian tubes, the walls are thrown into irregular longitudinal folds. These may be divided into two series, separated transversely by more flattened cells. The folds of the anterior series are made up of tall columnar epithelium with a small central core of connective tissue; while those of the posterior series consist of slightly lower cells resting on a well-marked basement membrane, and supported by a thick connective tissue cushion. There are no chitinous spines in this region.

Behind these folds is the tooth-bearing posterior gizzard. This shows longitudinal ridges which are lower than those of the preceding series, and bear large chitinous spines. The muscle-layers are thick, and as in *Mastotermes,* consist of inner oblique fibres, sometimes showing a fan-like arrangement beneath the spine-bearing cells, and outer longitudinal fibres. The posterior gizzard is shorter than in *Calotermes,* i.e., the tooth-bearing region is more concentrated. Folds similar to those of the second series lead from the gizzard to the large intestine.

The hind-gut of *Porotermes* seems to be leading on to the *Coptotermes* type. It differs from that of *Eutermes* in those points which have already been described for *Calotermes.*

3. Mesotermitidae.

Coptotermes flavus (Fig. 1e).—Between the entry of the Malpighian tubes and the posterior gizzard, the walls of the hind-gut are folded, and lined with columnar cells.

The posterior gizzard has well-marked teeth, and its walls have numerous irregular longitudinal folds. As in *Porotermes,* there is a definite demarcation between the toothless longitudinal folds and those of the posterior gizzard, and also, as in *Porotermes,*

there is a series of toothless folds between gizzard and large intestine. These probably correspond to the "four groups of columnar cells" which Imms describes as extending from the "pyloric valve" of *Archotermopsis* to the large intestine.

These three groups of termites, therefore, although showing considerable variation, resemble each other in general plan, and differ markedly from the Metatermitidae.

Discussion.

The alimentary canals of termites of different families can be arranged in a definite series, corresponding to their systematic positions. This series shows a progressive reduction of the spine-bearing area, together with elaboration of the folds in front of the posterior gizzard, and development of a further series of toothless folds behind it.

The most primitive termite, *Mastotermes,* has, as described above, an extensive spine-bearing area both anterior and posterior to the posterior gizzard. The folds at the beginning of division (*a*) of the hind-gut are relatively simple.

The comparatively unspecialized Protermitidae, *Archotermopsis* and *Calotermes,* closely resemble *Mastotermes.* In *Archotermopsis* the spine-bearing area anterior and posterior to the true gizzard is represented merely by a thickening of the chitinous lining. In *Calotermes* the posterior folds leading into the large intestine are very poorly developed, and there is no thickening of their chitinous lining, but the region anterior to the posterior gizzard retains its teeth. In both *Calotermes* and *Mastotermes* these anterior teeth point in the opposite direction to those of the gizzard.

Stolotermes, although its anterior folds are simple, shallow, and uniform, has no teeth except in the posterior gizzard. *Porotermes* also has its teeth confined to a short thick-walled gizzard, but the anterior folds are very well-developed, and are divided by a circular constriction into two series. There are also definite toothless folds behind the posterior gizzard.

Of the Mesotermitidae, *Coptotermes* very closely resembles *Porotermes* except that the folds behind the posterior gizzard are better developed.

In Metatermitidae such as *Eutermes,* the typical structures of the anterior part of the hind-gut are very much altered, and new parts are introduced. Thus the characteristic double valve may be regarded as a modification of the most anterior folds of other forms, correlated with the elongation of division (*a*) of the hind-gut. The glandular strip, histologically resembling mid-gut tissue, is a new development, as is the thin-walled tube which forms the hinder part of division (*a*).

Termites of this family are regarded by Imms, Tillyard, Wheeler, and others as the most highly-evolved Isoptera. They have usually no intestinal protozoa, although Cleveland (1926) has found a few small amoebae and flagellates in two species of *Amitermes*. Numerous spirochaetes and fungi are usually present. The fungus-gardens described by many authors are made only by termites of this family. Many Metatermitidae are grass-eating; others, however, are wood-feeding.

Although it is, so far, not possible to establish any definite relationship between the mode of nutrition and the structure of the alimentary canal, it is worthy of note that the extreme modifications of structure occur in those termites in which the digestive function of the alimentary canal must differ greatly from that of other groups.

Summary.

(1) The general external features of the alimentary canal in termites are described. The hind-gut can be divided into five regions, termed (*a*), (*b*), (*c*), (*d*), and (*e*), of which (*a*) and (*b*) are treated in some detail.

Division (*a*) is greatly elongated in the Metatermitidae.

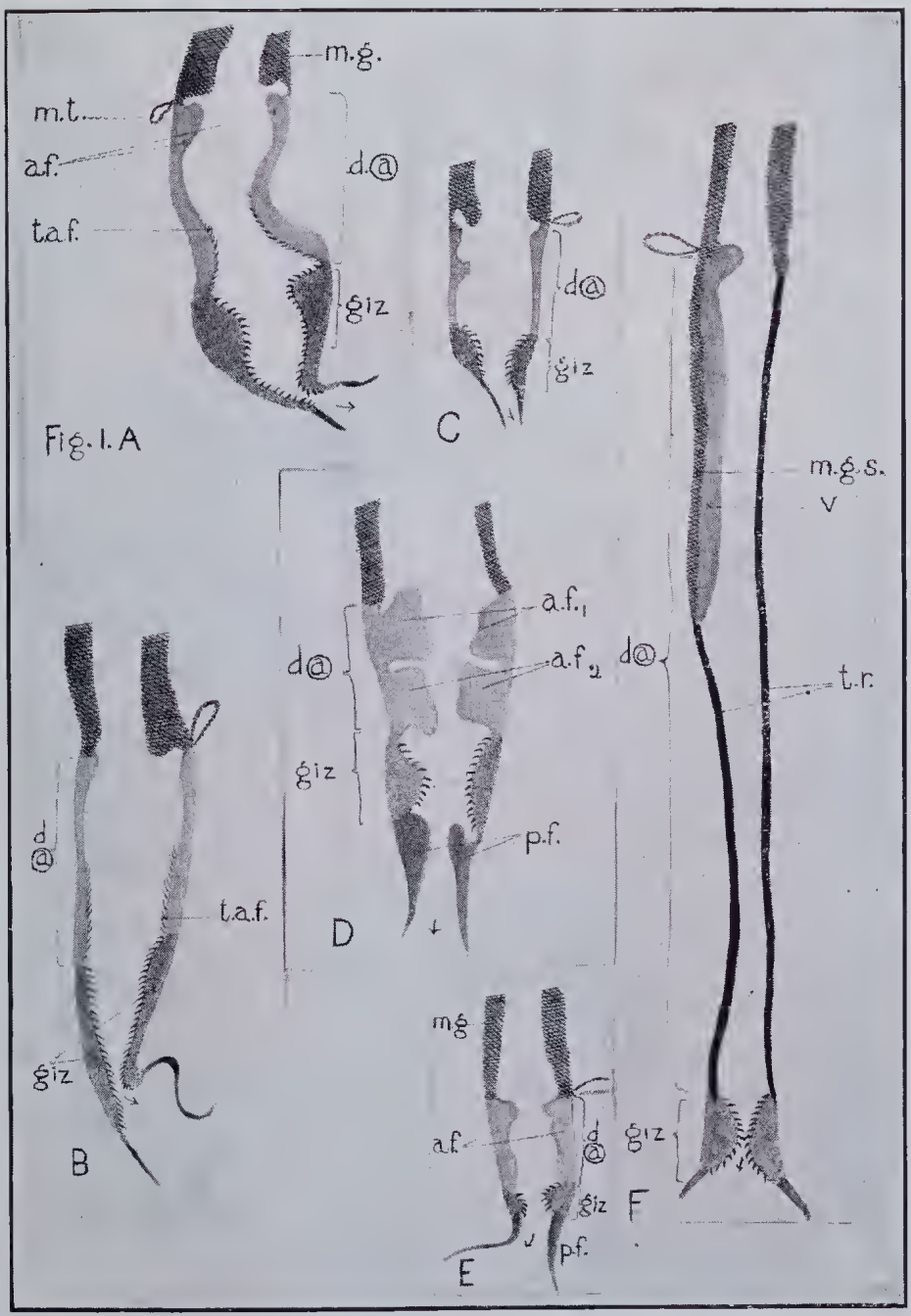
(2) Macroscopic features of the alimentary canals of *Eutermes dixoni* and *E. exitiosus* are described. These are very similar to *Eutermes chaquimayensis* as given by Holmgren (1909).

(3) The histology of the alimentary canal of *E. dixoni* and *E. exitiosus* is described, with particular reference to divisions (*a*) and (*b*) of the hind-gut.

Certain structures are present which were not mentioned by Holmgren. A strip of glandular cells similar to those of the mid-gut extends down one side of division (*a*) of the hind-gut. This strip has on each side of it a fold of epithelium forming a double valve; the folds almost surround the gut at their extreme anterior end, and fuse in the mid-line at the posterior. The rest of division (*a*), which is extremely elongated, is lined by flattened epithelium not seen in termites of other families. Division (*b*) bears chitinous spines, and the wall is deeply folded and very muscular. It has here been termed the posterior gizzard, and leads directly into division (*c*)—the large intestine.

(4) Brief descriptions of the histology of divisions (*a*) and (*b*) of the hind-gut of Mastotermitidae (*Mastotermes*), Protermitidae (*Calotermes, Stolotermes, Porotermes*) and Mesotermitidae (*Coptotermes*) are given. In main features these resemble each other, and differ greatly from Metatermitidae.

(5) The alimentary canals of termites of various families are arranged in series, corresponding to their systematic position. This series shows a reduction of the spine-bearing area, and an elaboration of the folds anterior and posterior to it.



Alimentary Canals of Termites.

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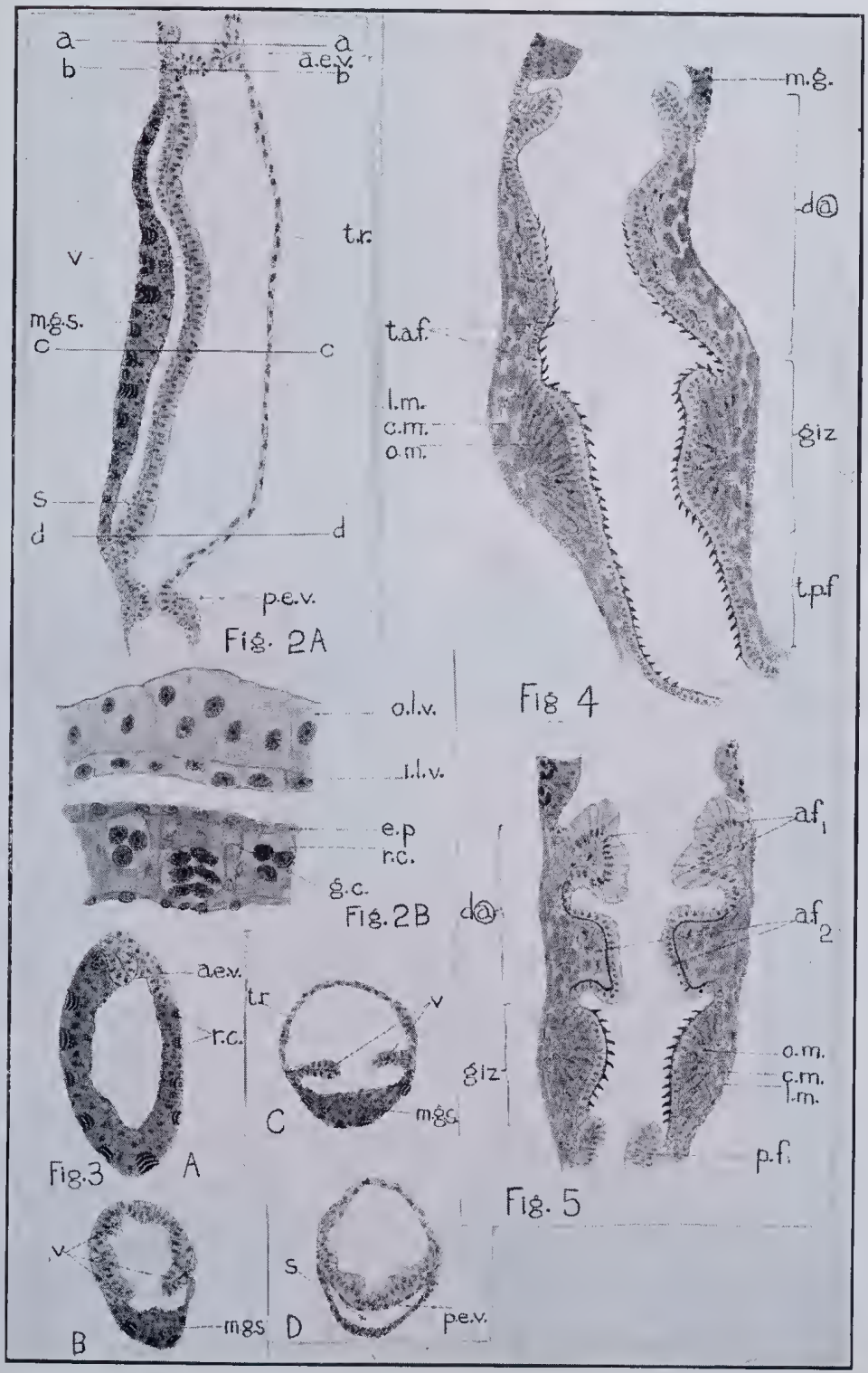

Alimentary Canals of Termites.

Eutermes shows the greatest modification of primitive structures, as shown in *Mastotermes*, and also the introduction of new ones. This is probably correlated with its lack of intestinal protozoa, which would imply a mode of nutrition different from that of other termite families.

In conclusion, I wish to thank Professor Agar for his supervision, and Dr. G. Buchanan for her help throughout the year. My thanks are also due to Mr. G. F. Hill, of the Council for Scientific and Industrial Research, who has supplied and identified most of my material.

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Explanation of Plates I. and II.

All outlines were drawn with the camera lucida.

Fig. 1.—Plan only; details of Figs. 2, 3, 4 and 5 were compiled from two or three adjacent sections.

Plate I.

Fig. 1.—Longitudinal sections of alimentary canals of various termites to show the demarcation of the different regions. (× 40.)
A. *Mastotermes;* B. *Calotermes;* C. *Stolotermes;* D. *Porotermes;* E. *Coptotermes;* F. *Eutermes.*

Plate II.

Fig. 2.—A., L. S. Alimentary canal of *Eutermes*. (× 66.)
a-a, b-b, c-c, d-d, levels of transverse sections in Fig. 3.
B., H. P. Portion of valve and mid-gut strip. (× 360.)

Fig. 3.—A, B, C, D. T. S. Alimentary canal of *Eutermes* at levels shown in Fig. AA. (× 66.)

Fig. 4.—L. S. Alimentary canal of *Mastotermes*. (× 66.)

Fig. 5.—L. S. Alimentary canal of *Porotermes*. (× 66.)

Lettering.

a.e.v. = anterior end of valve. a.f. = folds anterior to posterior gizzard. $a.f._1$ and $a.f._2$ = 1st and 2nd series of anterior folds. c.m. = circular muscle-fibres. d. a. = division a of hind-gut. e.p. = epithelium. g.c. = glandular cell. giz. = posterior gizzard. i.l.v. = inner layer of valve. l.m. = longitudinal muscle-fibres. m.g. = mid-gut. m.g.s. = strip of mid-gut like tissue. m.t. = malpighian tube. o.l.v. = outer layer of valve. o.m. = oblique muscle-fibres. p.e.v. = posterior end of valve. p.f. = folds behind posterior gizzard. r.c. = regenerative crypt. s. = space between valve and mid-gut strip. t.a.f. = tooth-bearing folds anterior to posterior gizzard. t.p.f. = tooth-bearing folds posterior to posterior gizzard. t.r. = thin-walled region opposite valve. v. = valve.